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L2: Entry 1 of 1 File: PGPB Nov 15, 2001

DOCUMENT-IDENTIFIER: US 20010042255 A1
TITLE: PRODUCTION OF BIOFILAMENTS IN TRANSGENIC ANIMALS

Pre-Grant Publication (PGPub) Document Number (1):
20010042255

Summary of Invention Paragraph (8):
[0006] In a third aspect, the invention features a female mammal in which the genome of the mammary tissue of the female mammal contains a nucleic acid molecule that includes (i) a nucleic acid sequence encoding a biofilament, (ii) a promoter that directs expression of a polypeptide in milk-producing cells, where the promoter is operably linked to the nucleic acid sequence, and (iii) a leader sequence that enables secretion of the biofilament by the milk-producing cells, into milk of the female mammal. Preferably, the female mammal is a rodent, a ruminant, or a goat.

Brief Description of Drawings Paragraph (2):
[0027] FIG. 1A is a schematic representation of the goat .beta.-casein/NcDS-1 construct containing the .beta.-casein promoter and signal sequence, the 1.5 kb NcDS-1 cDNA, and the 3' UTR from .beta.-casein.

Detail Description Paragraph (59):
[0072] In the following example, the design of the construct includes the use of the goat .beta.-casein promoter (Ebert et al., Bio/Technology 12: 699-701, 1993), followed by its own signal sequence for expression, followed by a 1.5 kb insert containing the silk clone (Arcidiacono et al., supra) in frame with the 5' and 3' ends of the casein gene. A schematic diagram of this construct is shown in FIG. 1A.

Detail Description Paragraph (60):
[0073] The nucleic acid molecule encoding a biofilament (e.g., a silk or fibroin gene, or fragment thereof) is fused to the casein promoter and secretion of the biofilament protein is driven by signal sequences from the gene from which the promoter is derived, or from the biofilament nucleic acid molecule to be expressed. Termination sequences can be derived from the biofilament gene itself, or from the promoter gene. Furthermore, a hybrid gene can be created to increase the level of expression. For this purpose, the silk or fibroin gene (or fragment thereof) can be inserted between exon 2 (just upstream of the ATG) and exon 7 (downstream of the stop codon) of the goat .beta.-casein gene (Ebert et al.,

Bio/Technology 12: 699-701, 1993). Since the highly repetitive nature of the construct raises concerns over the stability of the gene and the possibility of recombination due to the repetitive sequences, they can be disrupted by inserting the introns from the casein gene (introns 3 to 7). Construction of the vector can be performed in a cosmid vector (supercos), due to the large size of the final construct or the backbone of the plasmid could consist of any known bacterial vector (preferably ones that accept large DNA sizes) which contains sequences necessary for its amplification in an E. coli host (Sambrook et al., supra).

CLAIMS:

4. The mammal of claim 3, wherein said mammal is a selected from the group consisting of a rodent, a ruminant, and a goat.

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L4: Entry 1 of 1 File: PGPB Nov 15, 2001

DOCUMENT-IDENTIFIER: US 20010042255 A1
TITLE: PRODUCTION OF BIOFILAMENTS IN TRANSGENIC ANIMALS

Pre-Grant Publication (PGPub) Document Number (1):
20010042255

Detail Description Paragraph (45):
[0062] The transcription termination region of the nucleic acid constructs may involve the 3'-end and polyadenylation signal from which the 5'-promoter region is derived. For example, the bovine .alpha.S1 casein gene. Alternatively, the 3'-end of the nucleic acid construct will contain transcription termination and polyadenylation signals which are known to regulate post-transcriptional mRNA stability such as those derived from bovine growth hormone, .beta.-globin genes, or the SV40 early region.

Detail Description Paragraph (49):
[0065] The expression vectors used for the generation of transgenic animals may be linearized by restriction endonuclease digestion prior to transformation of a cell. In a variant of this method, only a digestion fragment that includes the coding, 5'-end regulatory sequences (e.g., the promoter), and 3'-end regulatory sequences (e.g., the 3' untranslated region) from, for example, bovine casein or growth hormone sequences, will be used to transform cells. A cell transformed with such a fragment will not, consequently, contain any sequences that are necessary solely for plasmid propagation in bacteria (e.g., the cell will not contain the E. coli origin or replication or a nucleic acid molecule encoding an antibiotic-resistance protein (e.g., an ampicillin-resistance protein) that is useful for selecting prokaryotic cells).

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L6: Entry 1 of 1 File: PGPB Nov 15, 2001

DOCUMENT-IDENTIFIER: US 20010042255 A1
TITLE: PRODUCTION OF BIOFILAMENTS IN TRANSGENIC ANIMALS

Pre-Grant Publication (PGPub) Document Number (1):
20010042255

Summary of Invention Paragraph (8):
[0006] In a third aspect, the invention features a female mammal in which the genome of the mammary tissue of the female mammal contains a nucleic acid molecule that includes (i) a nucleic acid sequence encoding a biofilament, (ii) a promoter that directs expression of a polypeptide in milk-producing cells, where the promoter is operably linked to the nucleic acid sequence, and (iii) a leader sequence that enables secretion of the biofilament by the milk-producing cells, into milk of the female mammal. Preferably, the female mammal is a rodent, a ruminant, or a goat.

Summary of Invention Paragraph (28):
[0026] By "transgenic" is meant any cell which includes a nucleic acid sequence that has been inserted by artifice into a cell, or an ancestor thereof, and becomes part of the genome of the animal which develops from that cell. Preferably, the transgenic animals are transgenic mammals (e.g., rodents or ruminants). Preferably the nucleic acid (transgene) is inserted by artifice into the nuclear genome.

Detail Description Paragraph (38):
[0058] Alternatively, for expression in the milk, for example, the promoter region may be native to a ruminant mammary-specific gene. Examples include: .alpha.S1-casein (PCT Application Nos.: WO91/08216 and WO93/25567), .alpha.S2-casein, .beta.-casein (Rosen, J. M., U.S. Pat. No. 5,304,489; Lee et al., Nucleic Acids Res. 16: 1027-1041, 1988), .kappa.-casein, .beta.-lactoglobin, and .alpha.-lactalbumin (Vilotte et al., Eur. J. Biochem. 186: 43-48, 1989;PCT Application No.: WO88/01648). These promoters can drive a high level of expression of a variety of proteins in a tissue and lactation specific manner.

Detail Description Paragraph (96):
[0091] Hence, mammalian (e.g., ruminant) zygotes are microinjected (or co-microinjected) with two nucleic acid fragments: one that expresses the biofilament protein under the control of a milk promoter, and one that expresses the biofilament protein under the influence of a urine specific promoter. The generated transgenic

animal will be secreting/producing the biofilament in both its milk and in its urine. This will increase the total output of biofilaments produced per transgenic animal unit.

CLAIMS:

4. The mammal of claim 3, wherein said mammal is a selected from the group consisting of a rodent, a ruminant, and a goat.

WEST Search History

DATE: Saturday, August 09, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB; PLUR=YES; OP=ADJ			
L8	((800/7)!.CCLS.)	71	L8
DB=PGPB; PLUR=YES; OP=ADJ			
L7	((800/7)!.CCLS.)	22	L7
L6	L1 and ruminant	1	L6
L5	L1 and cows	0	L5
L4	L1 and bovine	1	L4
L3	L1 and cow	0	L3
L2	L1 and (goat or goats)	1	L2
L1	20010042255	1	L1

END OF SEARCH HISTORY

- The specification does not specifically discuss making transgenic cows.

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L10: Entry 24 of 36 File: USPT Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780009 A
TITLE: Direct gene transfer into the ruminant mammary gland

Detailed Description Text (42):
If desired, the usefulness of a genetic construct can be estimated by introducing it into a cultured mammary epithelial cell line in vitro and assaying for production of the desired secreted protein, tethered enzyme, antisense oligonucleotide, or ribozyme. A particularly useful cell line for testing genetic constructs is the bovine mammary epithelial cell line termed MAC-T (U.S. Pat. No. 5,227,301, incorporated herein by reference). MAC-T cells display several features of differentiated mammary epithelia. For example, these cells feature apical microvilli and apical junctional complexes, imparting on the cells an apical-basal polarity. In addition, they form high resistance monolayers. In the presence of extracellular matrix and lactogenic hormones, MAC-T cells differentiate and secrete endogenous, mammary-specific proteins. If desired, MAC-T cells can be used for testing genetic constructs to be introduced into any of the ruminants, including constructs to be introduced into sheep and goats. Thus, these cells provide an art-recognized model system for ruminant mammary glands.

Detailed Description Text (88):
Results: Our results indicate that the use of a pJ7-hGH expression vector was an effective means for producing hGH within mammary cells in culture and within the mammary gland in vivo. Transiently transfected MAC-T cells routinely produced 7-10 ng/ml per 48 hours per 2.times.10.sup.5 cells. Non-transfected MAC-T cells did not produce detectable levels of hGH. These in vitro data demonstrated that the pJ7-hGH expression vector was correctly constructed and that it could direct the secretion of human growth hormone from bovine mammary epithelial cells.

Detailed Description Text (96):
Plasmid Construction: Plasmid pSFG-tPA included CDNA encoding human tPA under the transcriptional control of the Moloney Murine Leukemia Virus long terminal repeat promoter (MLV-LTR) as is shown in FIG. 3. The plasmid DNA was transfected into MAC-T cells using the lipofection protocols described above. The supernatants from transfected and non-transfected (control) cell cultures were assayed by ELISA for the presence of htPA. The amount of htPA present in the media 48 hours after transfection was approximately 40.3.+-.2.7 ng/ml. Detection of htPA in the media indicates that the genetic construct was able to direct the expression and secretion of htPA in bovine mammary epithelial cells.

Detailed Description Text (99):
Expression of Human Placental .beta.-galactosidase in MAC-T cells: To direct secretion of .beta.-galactosidase from bovine mammary epithelial cells, an expression vector was constructed which encoded human placental .beta.-galactosidase, a .beta.-galactosidase which contains a signal peptide. To assess the usefulness of this genetic construct, we assayed whether this protein could be secreted from MAC-T cells, the established model system for the ruminant mammary gland.

Detailed Description Text (101):
Plasmid pJ7-GP8 was transfected into MAC-T cells using the lipid-based technique described above. It is preferred that no .beta.-galactosidase activity from sources other than the genetic construct be present in the media. Accordingly, media containing heat-inactivated (1 hour at 60.degree. C.) fetal calf serum was used. The conditioned media from the transfected cells was harvested 72 hours after transfection and assayed for .beta.-galactosidase activity using the fluorogenic substrate 4-methylumbeliferyl .beta.-galactoside (Hubbes, et al., 1992, Biochem. J. 285:827). In this assay, it was determined that 139 nmol per minute of substrate was hydrolyzed by the .beta.-galactosidase in the supernatant of transfected cells. There was no detectable activity in the control supernatant from non-transfected cells. These data demonstrate that biologically active .beta.-galactosidase can be produced in, and secreted from, bovine mammary epithelial cells. This observation, in conjunction with the fact that the MAC-T cells represent a suitable model system for the ruminant mammary gland, indicates that genetic constructs which direct production of .beta.-galactosidase in milk can be successfully produced.